STUDIES ON INDOLE ALKALOID BIOSYNTHESIS. PART II. ALKALOIDS LACKING THE NORMAL TRYPTOPHAN SIDE CHAIN.

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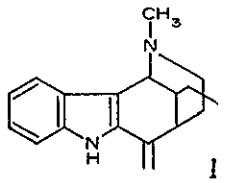
A summary of experiments relating to the biosynthesis of apparicine (II) and uleine (I) is presented. These alkaloids which lack the two carbon unit normally derived from tryptophan provide an interesting comparison with the Aspidosperma and Iboga bases for which a considerable amount of biosynthetic data has been accumulated. It is shown that tryptophan is utilized by the plant (Aspidosperma pyricollum) to provide the indole unit and the one carbon bridge between the β-position of the indole and the basic nitrogen atom in apparicine. Secodine is also incorporated intact into this alkaloid but the levels of incorporation are low. Interpretation of these results and their implications for future experiments are also provided.

Preliminary experiments relating to the biosynthesis of olivacine (III, R = H; R' = CH_3) and guatambuine (XV) are presented.

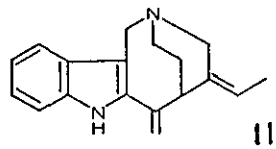
Part I[1] of this series provided a summary of our experiments as they pertain to the later stages of the biosynthesis of the Aspidosperma and

Iboga alkaloids. As mentioned in that review a large amount of experimental data has been obtained particularly in terms of the earlier phases of the biosynthetic pathways. There is little question about the two precursors, tryptophan and seco-loganin, in terms of their relative roles as the main building units for the corynantheinoid and, in turn, for the above-mentioned alkaloids. Such is not necessarily the case for other series of indole alkaloids even though many of these have received much attention from a structural and/or chemical point of view. It is the purpose of this article to provide a summary of our experiments in one of these areas, that is, alkaloids which lack the normal tryptophan side chain.

Uleine (I), apparicine (II), olivacine (III, R = H; R' = CH_3) and ellipticine (III, R = CH_3; R' = H) were the alkaloids selected for our consideration and study since analyses of their structures[2] provide an interesting comparison with the previously studied families. It is clear that the "non-tryptophan" units, particularly in uleine and apparicine, are identical with those normally considered in Strychnos alkaloid biosyn-



I



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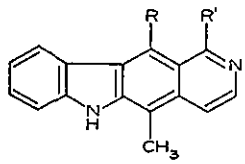


III

thesis thereby implicating an isoprenoid origin. The interesting portion of these alkaloids, in terms of biosynthetic considerations, is the indole unit. The two-carbon side chain of tryptophan which is readily apparent in the Aspidosperma, Iboga and Hunteria bases studied previously[1] is obviously absent in the above-mentioned alkaloids. Thus uleine is completely lacking these two carbon atoms, apparicine contains a one-carbon bridge between the β-position of the indole ring and the basic nitrogen atom while the olivacine-ellipticine series reveal three carbon atoms between this position of the indole ring and the basic heterocyclic nitrogen atom. It is therefore of interest to ask the following questions: a) Is tryptophan involved in the biosynthesis of the indole unit in these alkaloids? b) If not, what is the biosynthetic origin of this portion of these alkaloids?

Since we were successful in growing young plants of several Aspidosperma species[3] from which uleine and apparicine could be isolated, we directed our initial attention to these two alkaloids.

Wenkert[4] had already proposed a biosynthetic pathway for uleine in which he postulated that tryptophan was not involved but rather that an anthranilic acid derivative was being employed. He envisaged that glycosylideneanthranilic acid condensed with the seco-prephenate-formaldehyde (SPF) unit to yield an intermediate (IV) and the latter undergoes appropriate cyclizations via V → VI → VII to provide the alkaloid (Figure 1). At the time of the initiation of our studies the SPF unit had been discarded in favor of the structurally similar seco-loganin molecule derived from an isoprenoid pathway. This latter substance could however behave in

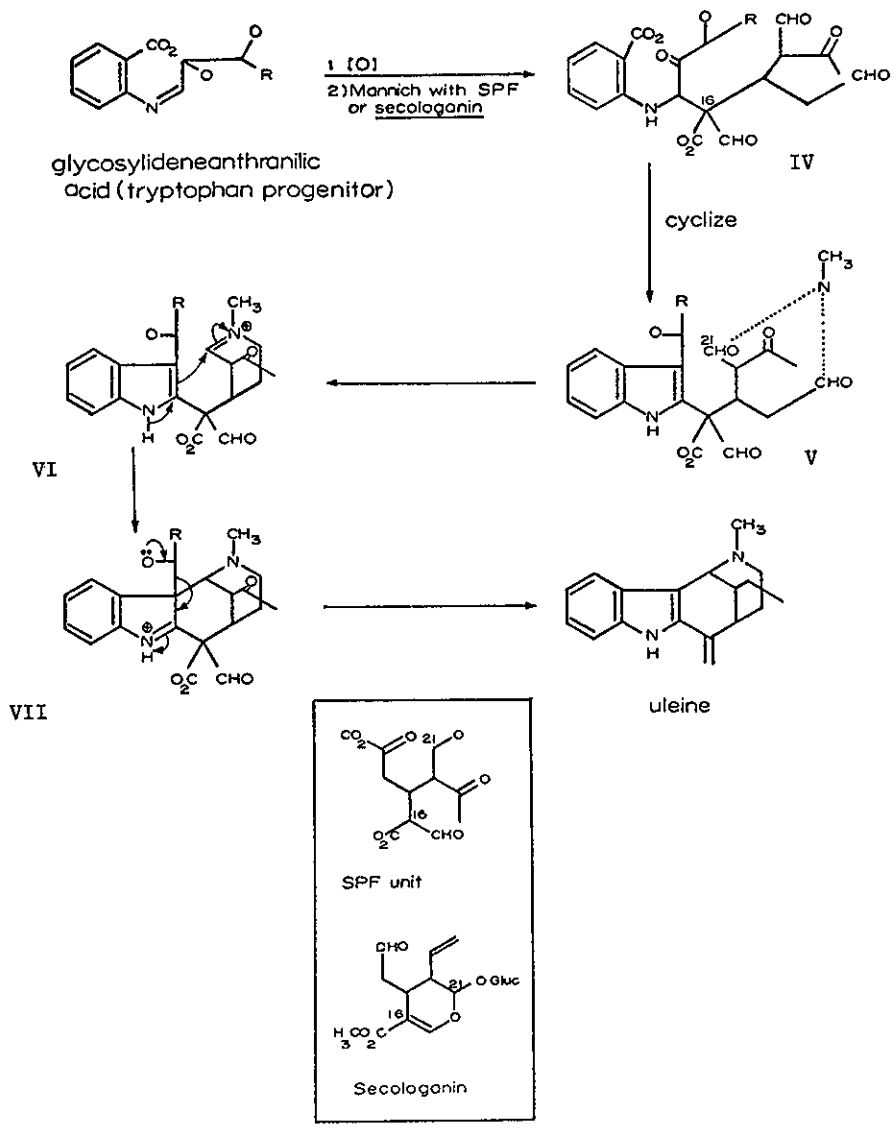


Figure 1. Wenkert's postulate for uleine.

a fashion similar to that of the SPF unit in terms of its condensation with the tryptophan progenitor so that the essential features of Wenkert's proposal, as they relate to our study, remain intact.

Several years later, Djerassi and Gilbert[5] published their results on the structure elucidation of apparicine and suggested that the Wenkert intermediate VI may be capable of interconversion via VIII (Figure 2) to apparicine.

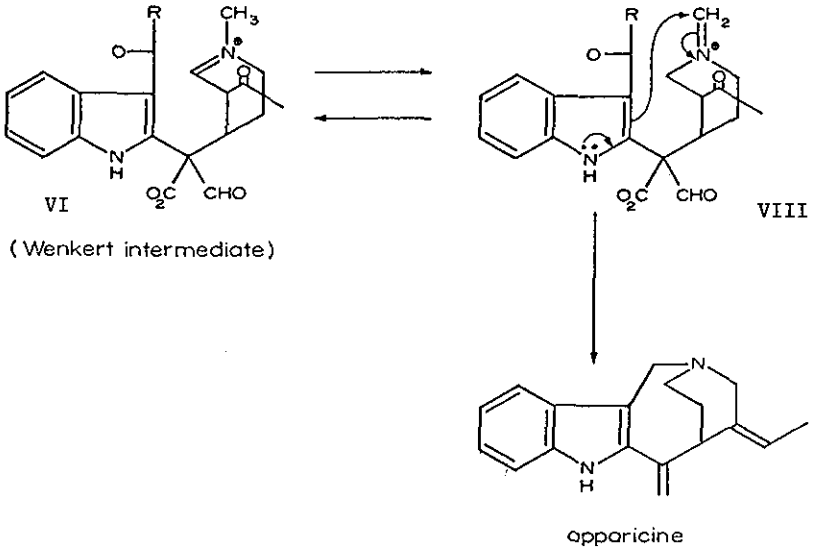


Figure 2. Djerassi-Gilbert postulate for apparicine.

In summary, the implications of the above postulates were: 1) The same precursor for both apparicine and uleine and 2) Neither the one-carbon (methylene) bridge which links the indole unit and the basic nitrogen atom in apparicine nor the N-methyl group of uleine are derived from tryptophan. Consequently these were the areas which we felt should receive initial consideration in our investigations.

Young plants (2 years old) of Aspidosperma pyricollum were initially investigated for alkaloid content, found to possess uleine and apparicine, and were selected for our study.

Initial biosynthetic experiments[6] in A. pyricollum plants utilized variously labelled forms of tryptophan in an attempt to obtain data pertaining to its biosynthetic role. In spite of numerous attempts with varying methods of incorporation, positive results with apparicine were consistently obtained but no activity could be detected in the isolated uleine. Table 1 provides a summary of the results obtained.

TABLE 1

Results of Incorporation of Double-labelled DL-Tryptophan into Aspidosperma pyricollum

Expt.	Label Distribution	Ratio of Activity ($^{14}C/^{3}H$)		
		Tryptophan Fed	Apparicine Isolated	Uleine Isolated
3.	Ar-^{3}H, $^{14}C_3$	1.1	1.5	Inactive
4.	Ar-^{3}H, $^{14}C_2$	1.0	<0.03	Inactive

The results shown in Table 1 indicate that tryptophan is incorporated into apparicine, that C_3 of this amino acid side chain is incorporated into the alkaloid and that over 97% of the activity at C_2 is lost during the biosynthesis.

Having demonstrated that tryptophan is involved, it was clearly of interest to determine at what stage in the biosynthetic pathway extrusion of this particular carbon atom occurs. For this purpose we prepared several intermediates, with tritium label in the aromatic ring, and proceeded to evaluate their role in this regard. Figure 3 summarizes the obtained results.[7]

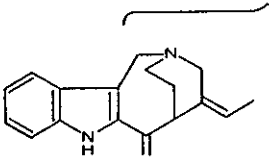
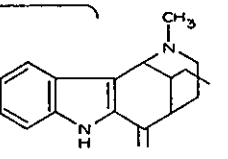
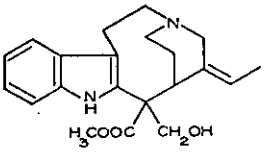
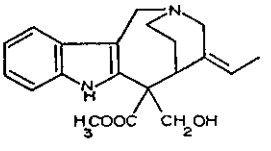
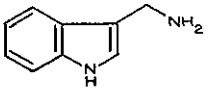
Expt.	Compd Fed	% Incorporation	
			
1	(ar-^{3}H) DL-Tryptophan	0.02	< 0.0001
2		0.55	< 0.0007
3		0.01	< 0.003
4		< 0.001	< 0.0001

Figure 3. Summary of results of incorporation of various intermediates into the alkaloids apparicine and uleine in A. pyricollum.

In terms of obtaining suggestive data as to possible extrusion of the original C_2 carbon atom of the tryptophan side chain at a late stage in the biosynthetic pathway, the incorporation of stemmadenine (expt. 2, Figure 3) was evaluated. As can be seen, the level of incorporation is the highest of any obtained in the investigations up to this time. For comparison, the alkaloid vallesamine[8] (expt. 3, Figure 3) which bears a much closer structural resemblance to appericine than stemmadenine, was also evaluated. Its level of incorporation was much lower than that obtained for stemmadenine.

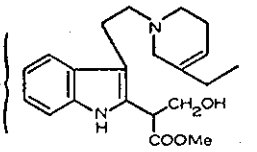
The simple indole derivative, 3-aminomethylindole (expt. 4, Figure 3) was evaluated as a possible intermediate which could suggest extrusion of C_2 at an early stage. This compound had already been considered as a precursor in the biosynthesis of gramine[9,10], a situation in which C_2 of the tryptophan side chain is also lost but C_3 is retained. As can be seen from Figure 3, no incorporation of this intermediate could be observed.

Clearly the results obtained thus far are insufficient to provide a very definitive pathway for the biosynthesis of apparicine but they at least suggest that stemmadenine or a closely related bio-intermediate is involved in the later stages of the pathway and thereby loss of the carbon atom from the original tryptophan side chain occurs at a late stage.

Our previous studies[1] had already employed secodinol and secodine, in variously labelled forms, in terms of their role as late stage bio-intermediates in the Aspidosperma and Iboga series so it seemed of interest to determine whether these substances can be utilized by A. pyricollum plants in the biosynthesis of apparicine. The details of the various experiments are published elsewhere[11] so it is appropriate here to simply provide

a summary of the pertinent results. Tables 2 - 4 illustrate the data obtained and provide a comparison with the other studies discussed previously.[1]

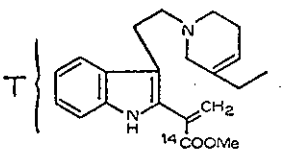
TABLE 2

Incorporation Studies with T 

Plant Species V. minor	Feeding Period	Feeding Method	Activity Fed	% Incorporation Minovine	% Incorporation Vincamine
1	24 hrs*	Hydroponic (acetate)	1.86×10^7 dpm	0	< 0.001
2	96 hrs*	"	2.40×10^7 dpm	< 0.001	< 0.001
V. rosea	216 hrs*	Wick	1.89×10^7 dpm	Vindoline	Catharanthine
				< 0.001	< 0.001
A. pyricollum	5 days	Hydroponic (roots)	9.20×10^7 dpm	Apparicine	Uleine
				< 0.001	< 0.001

*Marked plant deterioration after 24 hrs.

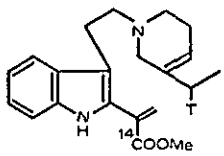
TABLE 3

Incorporation Studies with T 

Expt	Plant fed	Ratio of activity fed ($^3H/^{14}C$)	Ratio of activity isolated ($^3H/^{14}C$)	
1	V. rosea	8.8	Vindoline 8.3	
2	V. minor	8.4	Vincamine 8.6	Minovine 10.1
3	A. pyricollum	8.7	Apparicine 8.4	

TABLE 4

Incorporation Studies with.



Expt	Plant fed	Ratio of activity fed($^3H/^{14}C$)	Ratio of activity isolated ($^3H/^{14}C$)	
1	V. rosea	1.54	Vindoline 1.35	
2	V. minor	1.84,1.94	Vincamine 1.88, 2.02	Minovine
3	A.pyricollum	1.91	Apparicine 2.60	Uleine

As Table 2 reveals, secodinol is not incorporated into apparicine but the acrylic ester derivative secodine can be utilized by the plant (Tables 3 and 4). The ratios of activity ($^3H/^{14}C$) in the isolated apparicine obtained in these studies establish that the entire secodine molecule is being incorporated intact and that insignificant losses of the radiolabel occur during the various biotransformations.

Various degradations of the radioactive apparicine obtained were performed to establish the validity of the above statements. Figure 4 illustrates a degradation scheme which establishes that the ester group of the secodine molecule becomes the exocyclic methylene of apparicine while Figure 5 reveals the isolation of radioactive formaldehyde and

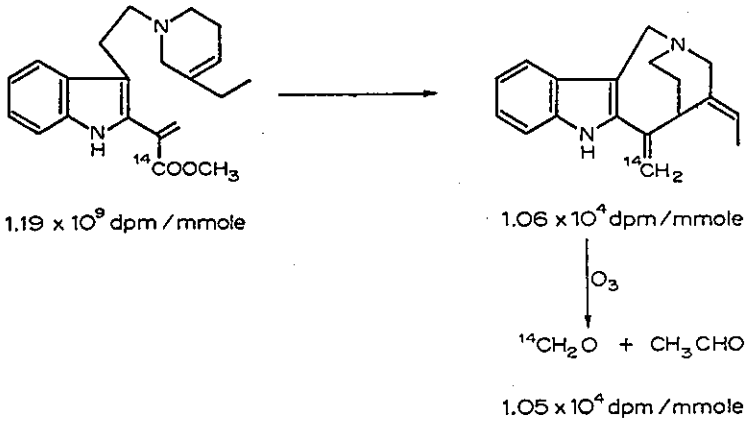


Figure 4. Degradation of apparicine from incorporation of [$^{14}COOCH_3$]-secodine.

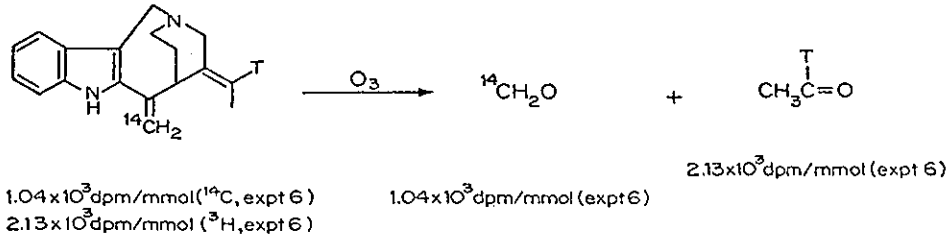
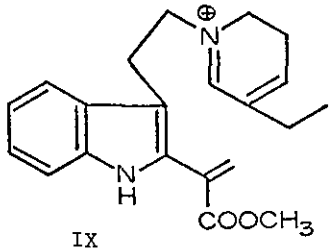


Figure 5. Degradation of apparicine from incorporation of [19-^{3}H, $^{14}COOCH_3$]-secodine.

acetaldehyde in an experiment in which a doubly labelled secodine had been incorporated into apparicine. In this latter case there was a significant loss of the tritium label (about 50%) situated in the ethyl side chain of the secodine molecule ($^3H/^{14}C$ drops from 3.98 in secodine

to 2.05 in the isolated alkaloid). This situation is not unexpected since the synthetic method employed for introduction of this label is expected to place the tritium atom in both R and S configurations. If the enzymic elaboration of this side chain to the ethylidene side chain in appariciné was to proceed in a stereospecific fashion only a partial loss of label would occur.

In a further series of experiments, secodine labelled with tritium in the piperideine ring, i.e. [3,14,15,21-^{3}H, $^{14}COOCH_3$)-secodine, was evaluated. Isolation of the radioactive appariciné and analysis of the $^{3}H/^{14}C$ ratio revealed significant loss of tritium during the conversion of secodine to the alkaloid (reduction in ratio from 4.2 to 2.2). These results suggest that a secodine derivative possessing a higher oxidation state, for example a dehydrosecodine (IX), may be involved in whatever conversions occur between secodine and appariciné. Obviously such speculations require further substantiation by appropriate experiments.



IX

At this point in time it is possible to summarize the results concerned with secodine incorporation as shown in Figure 6. It is also appropriate to summarize the other results obtained earlier in terms of a working hypothesis which provides a reasonable rationale and consideration for future studies.

Figure 6. A summary of results involving incorporation of secodine into appariçine in A. pyricollum.

As already mentioned earlier one of the most interesting features of the results as shown in Figure 3 is the high level of incorporation of stemmadenine thereby suggesting that this alkaloid, or a closely related intermediate, may be of crucial importance in the biosynthesis of apparicine. In the conversion of stemmadenine to apparicine it is obvious that two basic transformations are involved - conversion of the functionality at C_3 to the olefinic linkage and loss of C_8, the latter obviously being derived from the C_2 carbon atom of the tryptophan side chain. Clearly a fragmentation of the C_8-C_9 bond with subsequent extrusion of C_8 and recyclization to generate the one-carbon bridge present in apparicine must be accommodated in some mechanistic rationale. An attractive proposal to account for the overall process was put forward by a French group[12] (Figure 7). This postulate involves fragmentation of the C_8-C_9 bond in stemmadenine via a modified Polonovski reaction (X → XI), loss of C_8 in a hydrolytic process and cyclization of the intermediate XII thus formed to the alkaloid. Although this interesting proposal does provide a plausible rationale for the desired biotransformation it is not entirely consistent with the data which we have accumulated in this area. As already noted above, the carbomethoxy group of secodine is retained as the exocyclic

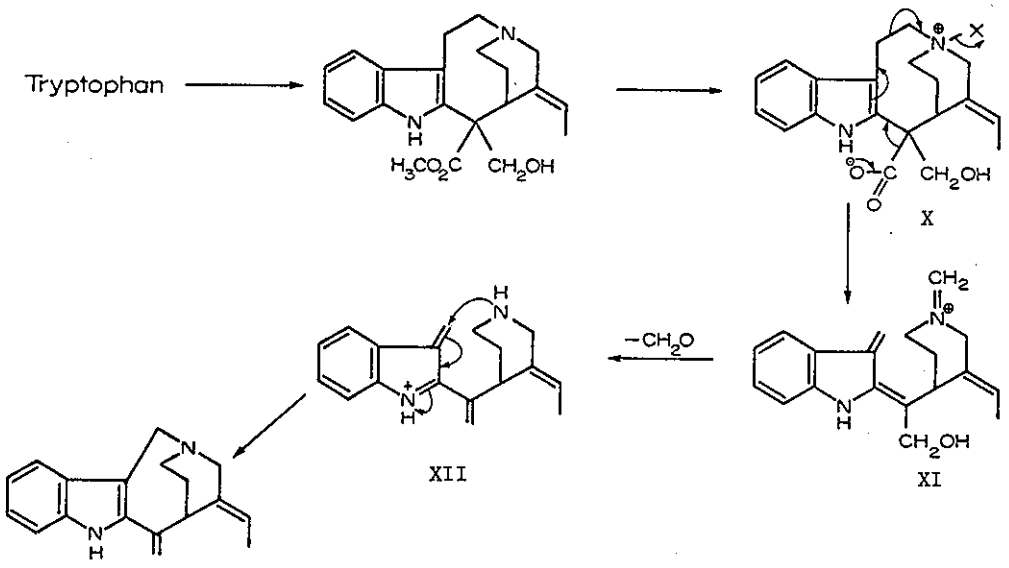


Figure 7. The Potier postulate for the conversion of stemmadenine to apparicine.

methylene in apparicine so it is logical to assume that the ester functionality in stemmadenine would be retained provided that a direct biological relationship exists between secodine and stemmadenine. The postulate as summarized in Figure 7 suggests a loss of the ester group via a concerted process in which decarboxylation and fragmentation occurs simultaneously. We believe that a Polonovski type fragmentation is indeed an attractive process for the stemmadenine-apparicine conversion and suggest a modification in which the loss of the hydroxymethylene group occurs (Figure 8). The French group has also put forward a modification to their original hypothesis.[13]

We also believe that our results with regard to the secodine incorportions are explicable in terms of a biological relationship between

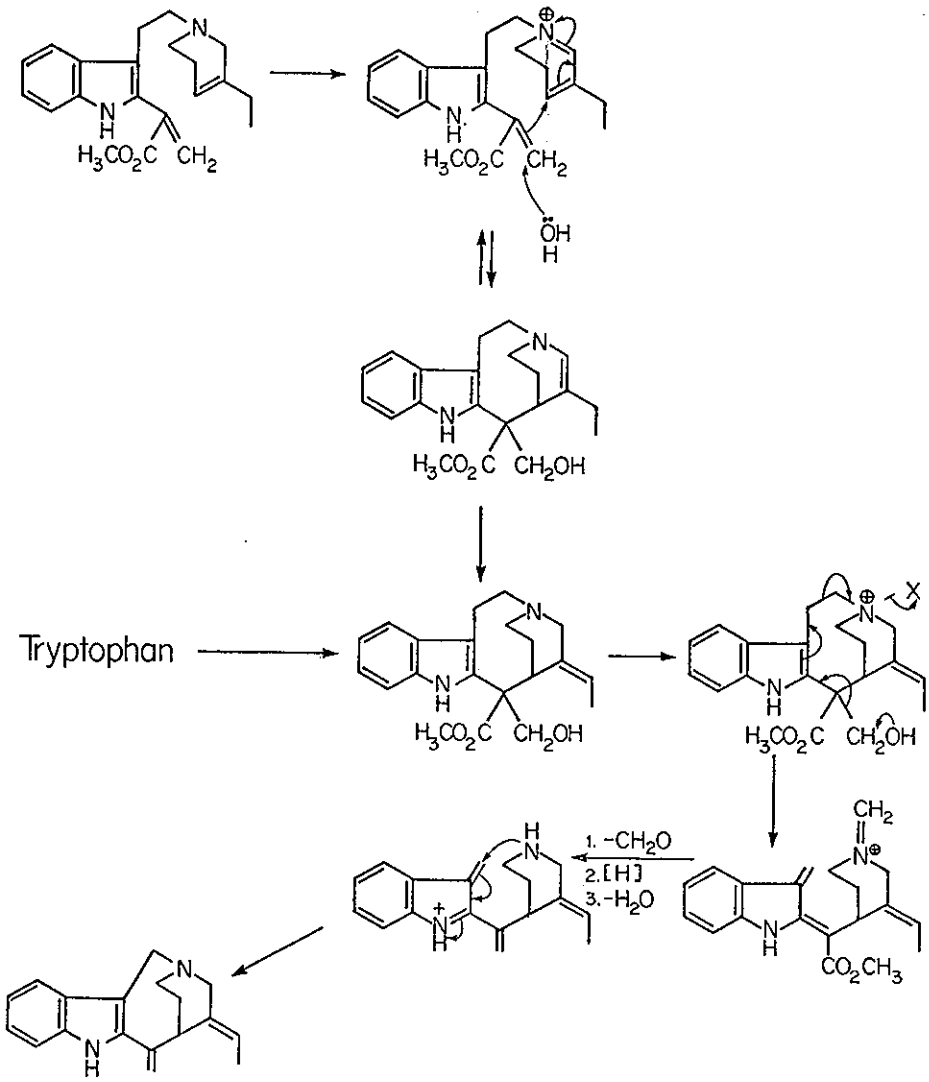


Figure 8. A postulate which is consistent with our experiments involving incorporation of tryptophan, stemmadenine and secodine into apparicine in A. pyricollum.

stemmadenine and the secodine system as shown in Figure 8. Thus secodine undergoes oxidative transformation to a dehydrosecodine and the latter then cyclizes to the stemmadenine system. This postulate is consistent with all the results obtained thus far.

A further understanding of the biosynthetic steps as they relate particularly to the later stages in this as well as the Aspidosperma and Iboga bases discussed in Part I[1] requires a detailed evaluation of stemmadenine and/or related Strychnos bases in terms of their biosynthetic role. The availability of stemmadenine is low and the opportunity for introduction of the appropriate label at various sites in these molecules is somewhat restricted. For this reason we have expended considerable effort in the development of a synthetic pathway leading to the akuammicine and stemmadenine series.[14] We believe that the sequence, as summarized in Figure 9, will allow ample opportunity for preparing the required radioactive analogues for further biosynthetic studies.

Difficulties in obtaining any meaningful data on the alkaloid uleine in A. pyricollum plants caused us to turn our attention to a related plant system, A. australe, which provided not only an alternative source of this alkaloid but also the opportunity for investigating the biosynthesis of the interesting family of alkaloids exemplified by olivacine (III, R = H, R' = CH_3) and guatambuine (XV). Experimental data relating to the latter family are completely lacking and this plant[3] appeared to be a good source of such alkaloids.

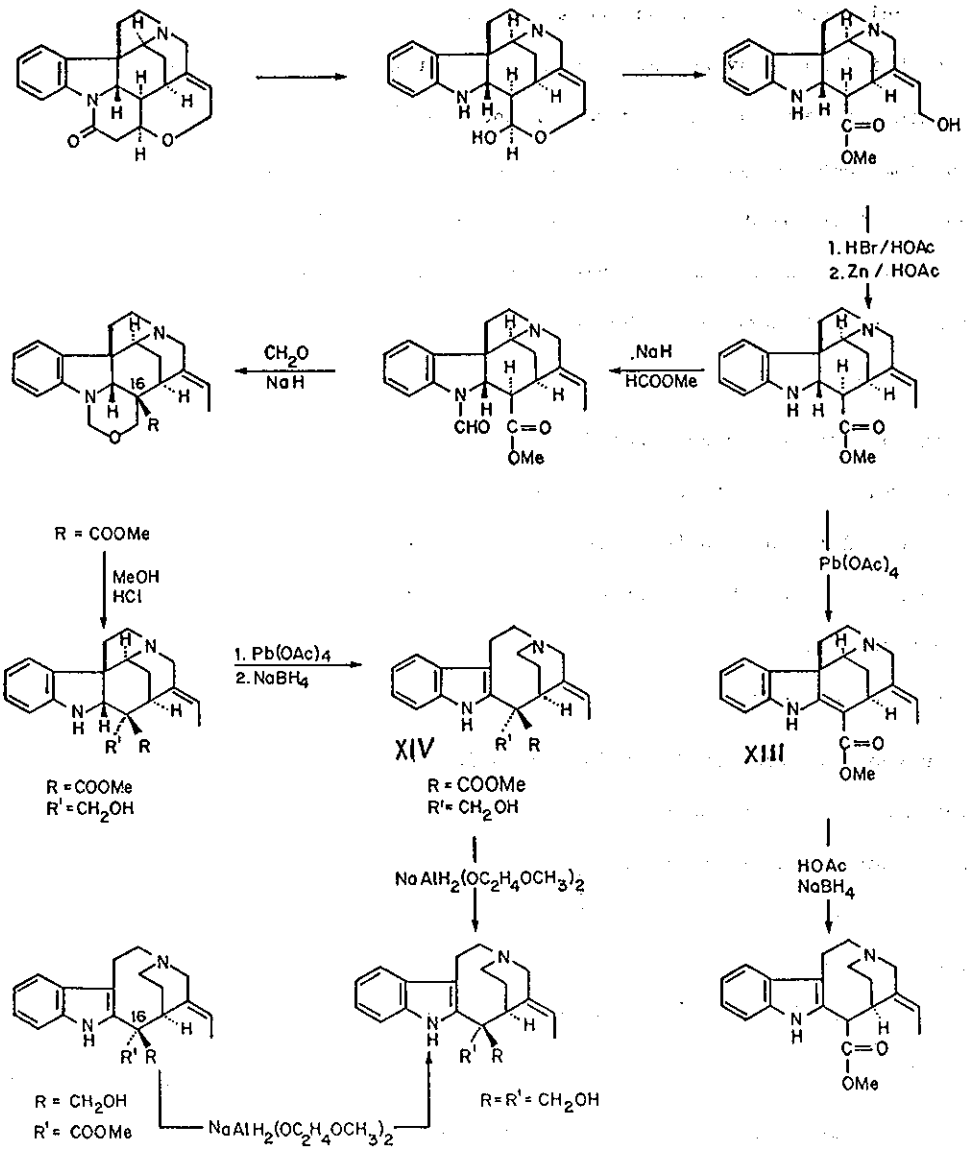


Figure 9. The total synthesis of akuammicine (XIII) and 16-epi-stemmadenine (XIV).

Speculations on the biosynthesis of olivacine and ellipticine were put forth initially by Wenkert[4] while a more recent and more detailed postulate has been advanced by Potier and Janot[13] (Figure 10). As is noted from these proposals tryptophan and stemmadenine are implicated so it became of interest to evaluate their roles in the biosynthetic pathway. Since stemmadenine was unavailable at this time, secodine was evaluated in its place. A summary of the various experiments are provided in Tables 5 and 6.

The precursors to be studied were fed hydroponically as their acetate salts to whole A. australe plants for 5 days. The crude alkaloidal extract was diluted with inactive samples of the desired alkaloids, which were re-isolated by thin layer chromatography and recrystallized to constant activity. A few comments concerning the results as shown in Tables 5 and 6 are now in order.

In general, low levels of incorporation, particularly into the alkaloids uleine and guatambuine were observed and it is difficult to make any definitive comments with regard to these alkaloids. In the case of uleine (experiment 3), C_2 of tryptophan seems to be retained since the ratio $^3H/^{14}C$ in the isolated alkaloid is identical with that incorporated (3.0 vs. 2.97). Obviously further substantiation of this result is required in an experiment where higher levels of incorporation are obtained.

Experiment 1 provided an indication that olivacine and guatambuine might be derived from tryptophan. Experiments 3 - 5 involving doubly labelled tryptophan resulted in either erratic ratios or extremely low

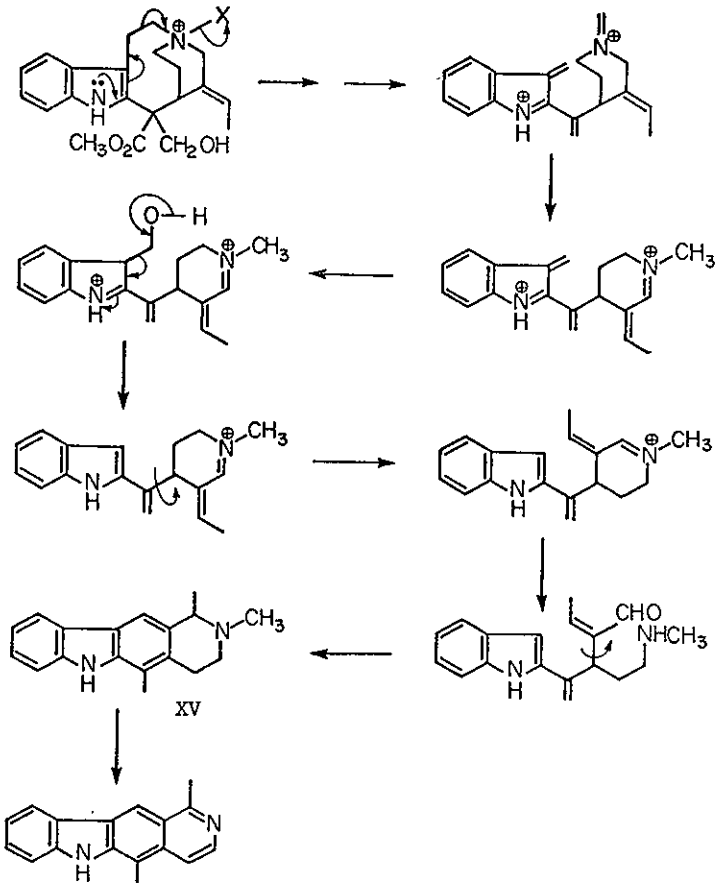


Figure 10. The Potier-Janot postulate for the biosynthesis of olivacine and guatambuine.

TABLE 5

Summary of Various Compounds Fed to A. australe Plants

Expt.	Compound Fed	Activity Fed ^{14}C dpm	Activity Fed ^{3}H dpm	Ratio Fed $^{3}H/^{14}C$
1	Tryptophan-(Ar-^{3}H)		8.28×10^{7}	
2	Secodine-(Ar-^{3}H, $^{14}COOCH_3$)	3.0×10^{7}	8.7×10^{7}	2.9
3	Tryptophan-(Ar-^{3}H, 2-^{14}C)	6.25×10^{7}	1.90×10^{8}	3.0
4	Tryptophan-(Ar-^{3}H, 3-^{14}C)	8.45×10^{7}	2.97×10^{8}	3.5
5	Tryptophan-(Ar-^{3}H, 2-^{14}C)	1.33×10^{8}	4.18×10^{8}	3.15
6	Secodine-(3,14,15,21-^{3}H, $^{14}COOCH_3$)	1.09×10^{7}	2.19×10^{7}	2.01

levels of radioactivity in the alkaloids isolated. If the postulate for the biosynthesis of olivacine and guatambuine (Figure 10) is correct, C_2 of tryptophan should be lost and the ratios of $^{3}H/^{14}C$ activity in experiments 3 and 5 do show an increase in the case of olivacine. Again further experimentation is necessary to confirm these findings. The increase observed for apparicine had already been established in the A. pyricollum plant system as discussed earlier.

Experiment 4, as it relates to olivacine, is inconclusive since the activity in the isolated alkaloid could not be achieved to a constant level and the value for the ratio isolated is only an approximation.

TABLE 6

Incorporation Results Associated with Table 5

Expt.	Alkaloid Isolated	Specific Activity Isolated (dpm/mmol) ^{14}C	^{3}H	% Incorporation ^{14}C	^{3}H	Ratio Isolated $^{3}H/^{14}C$
1	Olivacine	---	2.01×10^{7}	--	.241*	--
	Apparicine	---	2.0×10^{8}	--	.208	--
	Uleine	---	5.19×10^{5}	--	<.001	--
	Guatambuine	---	4.28×10^{6}	--	.005	--
2	Olivacine	2.66×10^{6}	1.04×10^{7}	.009*	.013*	3.90
	Apparicine	1.36×10^{7}	2.75×10^{7}	.039	.027	2.04
	Guatambuine	3.35×10^{5}	7.55×10^{5}	<.001	<.001	2.25
3	Olivacine	2.40×10^{5}	1.72×10^{6}	.0005	.001	7.18
	Apparicine	6.89×10^{5}	1.06×10^{8}	.0015*	.078*	154
	Uleine	7.42×10^{5}	2.14×10^{6}	.001	.001	2.97
	Guatambuine	8.24×10^{5}	3.34×10^{6}	.0015	.002	4.06
4	Olivacine	1.63×10^{5}*	9.09×10^{5}*	<.001	<.001	5.57
	Apparicine	9.0×10^{7}	2.64×10^{8}	.127	.105	2.90
5	Olivacine	1.05×10^{5}*	7.24×10^{5}*	<.001	<.001	6.90
6	Olivacine	8.34×10^{6}	1.85×10^{7}	.146*	.160*	2.22
	Apparicine	1.67×10^{5}	4.17×10^{5}	.004*	.005*	2.50

* not constant activity

Again, as in the studies involving A. pyricollum, C_3 of tryptophan is incorporated without loss into apparicine.

Experiments 2 and 6, involving secodine as the precursor, yielded no definitive data.

In conclusion, the results in A. australe are only preliminary and require substantiation. Obviously stemmadenine, in variously labelled forms, becomes a prime candidate for evaluation in the biosynthesis of olivacine and guatambuine as well as apparicine. Experiments in this direction are anticipated.

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